Induction of Interleukin-6 Production by Ultraviolet Radiation in Normal Human Epidermal Keratinocytes and in a Human Keratinocyte Cell Line is Mediated by DNA Damage

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The sunburn reaction is the most common consequence of human exposure to ultraviolet radiation (UVR), and is mediated at least in part by interleukin-6 (IL-6). The aim of this study was to determine if DNA is a major chromophore involved in the induction of IL-6 following UV irradiation of a human epidermoid carcinoma cell line (KB), and of normal human epidermal keratinocytes. We first confirmed that IL-6 release was associated with enhanced levels of IL-6 mRNA transcripts. The wavelength dependence for IL-6 release was then investigated by irradiating the cells at defined wavelengths (254, 302, 313, 334, and 365 nm) with a monochromator. The maximum effect on IL-6 release was observed at 254 nm with only low levels of induction observed at wavelengths above 313 nm. The wavelength dependence for UV-induced IL-6 release was similar to that for DNA absorption or for the induction of cyclobutane pyrimidine dimers (CPD). To determine whether UV-induced DNA damage mediated IL-6 secretion, the role of CPD was investigated by treating keratinocytes with photosomes (photolyase encapsulated in liposomes) followed by photoreactivating light. This photoreversal procedure led to a reduction in the levels of the UVC-induced secretion of IL-6, which in normal human keratinocytes was unambiguously associated with repair of CPD. We conclude that the release of IL-6 from human keratinocytes following short-wave UVC and UVB irradiation is mediated by DNA damage and that CPD play an important role in this process. ***Key words: cyclobutane pyrimidine dimers/DNA damage/interleukin-6/keratinocytes/(6–4) photoproduct/ultraviolet radiation. J Invest Dermatol 111:354–359, 1998***

Ultraviolet B (UVB, 280–315 nm) radiation is the shortest wavelength component of sunlight to reach the earth's surface. Exposure to solar UVB radiation is a significant human health risk that may be exacerbated by stratospheric ozone depletion (Slaper *et al*, 1996). It is well established that an initial step in UV-induced photocarcinogenesis is the formation of potentially mutagenic DNA damage in skin cells. The major types of damage induced directly by UV occur predominantly at dipyrimidine sites and include the cyclobutane pyrimidine dimer (CPD), the pyrimidine(6–4)pyrimidone photoproduct, and its Dewar isomer (Matsunaga *et al*, 1993; Sage, 1993; Clingen *et al*, 1995a). Other types of DNA damage, mainly oxidative forms, can be induced indirectly via alternative chromophores, and may predominate at longer wavelengths. In addition, UVB may induce changes in the expression of many genes (Keyse, 1993) and trigger the release of various cytokines from human keratinocytes [e.g., interleukin (IL)-6, TNFα, IL-1, IL-10, and IL-12] (Köck *et al*, 1990; De Vos *et al*, 1994; Grewe *et al*, 1995; Enk *et al*, 1996). These cytokines not only can be involved in local inflammatory or immunosuppressive reactions, but may also enter the circulation and have systemic effects.

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Abbreviations: CPD, cyclobutane pyrimidine dimers; NHEK, normal human epidermal keratinocyte; PRL, photoreactivating light; PS, photosomes.

The most common response experienced following human exposure to solar UVB radiation is the sunburn reaction. This delayed reaction involves the induction of several genes and is mediated, at least in part, by the release of IL-6 into the circulation from epidermal cells (Urbanski *et al*, 1990). In addition, an increase of IL-6 levels in serum is believed to be a general defense mechanism against injury before the onset of the immune response and is associated with the development of fever and the synthesis of acute phase proteins by the liver (Gauldie *et al*, 1987; Nijsten *et al*, 1987; Urbanski *et al*, 1990).

For ultraviolet radiation (UVR) to have a biologic effect it must first be absorbed by a cellular chromophore that translates the energy into a biochemical signal. A number of important chromophores have been identified and include porphyrins, flavins, steroids, aromatic amino acids, urocanic acid, and DNA. Membrane damage following UVR can also affect signal transduction pathways. Cell enucleation studies or UVB irradiation of cytosolic extracts have shown that UV activation of the transcription factor, nuclear factor kappa B, is membrane dependent (Devary *et al*, 1993; Simon *et al*, 1994). For the activation of the c-Jun amino-terminal protein kinase (JNK) in Hela cells, clustering of cell surface growth factor and cytokine receptors is required (Rosette and Karin, 1996). In addition to a role for DNA damage in UV-induced signal transduction pathways, DNA damage has been shown to act as a trigger for UV-induced immunosuppression. Evidence, derived primarily from murine studies, indicates that CPD in particular play an important role in mediating UVB-induced immune responses, initiating the production of immunosuppressive cytokines in murine keratinocytes and affecting antigen presentation of dendritic

cells (Kripke *et al*, 1992, 1996; Nishigori *et al*, 1996; Yarosh and Kripke, 1996; Vink *et al*, 1997).

Because the studies outlined above have focused primarily on animal models, the aim of this study was to investigate whether there was a link between UV-induced DNA damage and cytokine production in human keratinocytes. The wavelength dependence for IL-6 production from normal human epidermal keratinocytes and the epidermoid carcinoma cell line, KB, has been established. The role of CPD has been investigated by treating cells with photolyase encapsulated in liposomes (photosomes) in combination with photoreactivating light (PRL, 300–600 nm). CPD, but no other forms of DNA damage, can be monomerized by the repair enzyme photolyase using this procedure.

MATERIALS AND METHODS

Cell culture KB cells (ATCC, Rockville, MD) were maintained in monolayer culture in Eagle's minimum essential medium (MEM) with 20 U penicillin per ml, 20 μg streptomycin per ml, 2 mM glutamine, and 10% fetal calf serum (MEM-FCS 10%) (Gibco, Paisley, U.K.). This cell line was derived from an oral carcinoma of an adult male and has been used as a model system to study UVB-induced cytokine expression (Kirnbauer *et al*, 1989; Köck *et al*, 1990; Grewe *et al*, 1995). Normal human epidermal keratinocytes (NHEK) derived from neonatal foreskin were obtained from Clonetics (San Diego, CA) and were maintained in complete keratinocyte basal medium (Clonetics) supplemented with 0.1 ng epidermal growth factor per ml, 5 μg insulin per ml, 0.5 μg hydrocortisone per ml, 30 μg bovine pituitary extract per ml, 50 μg gentamycin per ml, and 50 ng amphotericin B per ml (KBM+). Both culture systems were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. For irradiation, 8×10^4 KB cells in 1 ml MEM-FCS 10% or 2×10^5 NHEK cells in 1 ml KBM+ without hydrocortisone (KBM–) were incubated overnight in tissue culture grade 3.5 mm Petri dishes (Nunc, Roskilde, Denmark). Prior to UV irradiation, cells were washed and medium replaced with 1 ml of PBS. Immediately after irradiation, 0.75 ml of MEM-FCS 10% or KBM-containing 1% heat-inactivated fetal calf serum, as appropriate, was added to each dish. Cells were cultured for up to 24 h at 37°C in a humidified atmosphere containing 5% CO_2. Supernatants were then collected, centrifuged at $600 \times g$, and stored at –20°C until determination of cytokine concentrations. At the same time, cells from each individual dish were trypsinised and the number of live cells evaluated using trypan blue.

UV irradiation The source of monochromatic UV irradiation was a 1/4 m Oriel monochromator with a 1 kW mercury-xenon arc lamp with 5 nm total bandpass slits and focused through a 5 mm × 1 m liquid light guide. Irradiance was determined with a UVX radiometer (UV Products, San Gabriel, CA) and sensors calibrated to a response of 1.0 for 1 mW per cm^2 at 254, 310, and 365 nm with instruments whose accuracy is traceable to the United Kingdom National Physical Laboratory. For the irradiation at 302 nm and 334 nm, irradiance was determined from the absolute response curve of the 310 nm UVX sensor. Irradiance was 0.79 W per m^2 at 254 nm, 9.12 W per m^2 at 302 nm, 15.9 W per m^2 at 313 nm, 5.12 W per m^2 at 334 nm, and 24.2 W per m^2 at 365 nm. Scattered short wavelength light did not contribute significantly to cytokine induction following 302 nm or 313 nm irradiation. Thus almost identical induction of IL-6 was observed at equivalent doses after irradiation at 302 and 313 nm through 2 mm, 2 inch square Schott long pass filters (H.V.SKAN, Solihull, U.K.) with 50% cutoff at 280 nm and 295 or 305 nm, respectively (results not shown).

IL-6-enzyme-linked-immunoassay Ninety six-well immunoplates (Nunc) were coated overnight at room temperature with mouse anti-human IL-6 antibody (1 μg per ml, 100 μl per well) (R&D Systems, Abingdon, U.K.). After this and each subsequent step, plates were washed four times with PBS containing 0.05% Tween 20 (Sigma, St. Louis, MO). To block nonspecific binding, 10% fetal calf serum, 200 μl per well was added for 1 h at 37°C. Serial dilutions of IL-6 standards (R&D Systems) or the supernatants to be tested were incubated for 2 h at room temperature in the 96 well plates. Subsequently, plates were incubated at ambient temperature with 100 μl per well of biotinylated goat anti-human IL-6 (100 ng per ml) (R&D Systems) and peroxidase conjugated streptavidin (1 μg per ml) (Jackson Immunoresearch Labs, West Grove, PA) for 2 h and 25 min, respectively. Finally, 100 μl of TMB (3,3′,5,5′ tetramethylbenzidine) peroxidase substrate solution (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well. The reaction was stopped after 10–15 min by addition of 100 μl per well of 1% sulfuric acid. Optical densities were read at 450 nm using a Multiskan MS plate reader (Life Sciences, Basingstoke, U.K.). The concentrations of IL-6 in the test samples were determined against standard curves using Genesis software (Life Sciences). The threshold of detection of this assay was 15 pg per ml. No cross-reactivity was observed with other known cytokines including IL-1 and TNFα.

Reverse transcriptase-polymerase chain reaction (RT-PCR) Cytoplasmic RNA was isolated using the guanidium thiocyanate phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). The amount of RNA extracted was estimated by spectrophotometry.

For cDNA synthesis 1 μg RNA, 25 units of MMuLV reverse transcriptase (Advanced Biotechnologies, Epsom, U.K.) and 100 ng of oligo dT were incubated at 37°C for 60 min in a final volume of 20 μl. To ensure that similar amounts of cDNA were used for PCR, expression of IL-6 and of the "housekeeping" gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were analyzed in the same sample. The cDNA was then diluted to a final volume of 50 μl. Diluted cDNA (5 μl) was amplified in the presence of 0.5 U Taq Polymerase per μl (Advanced Biotechnologies) and 25 pmol of both the sense and the anti-sense IL-6 or GAPDH oligonucleotides in PCR buffer (10 mM Tris pH 9, 50 mM KCl, 1,5 mM $MgCl_2$, 0.01% gelatin, and 0.1% triton X100):

IL-6 sense, 5′-GTACATCCTCGACGGCATCTCAGC-3′;
IL-6 anti-sense, 5′-TGTGGTTGGGTCAGGGGTGGTTAT-3′;
GAPDH sense, 5′-CCACCCATGGCAAATTCCATGGCA-3′;
GAPDH anti-sense, 5′-TCTAGACGGCAGGTCAGGTCCACC-3′.

The reaction mixture was incubated for 5 min at 94°C and then amplified by 33 PCR cycles (denaturation for 30 s at 94°C, annealing for 1 min 30 s at 65°C, and extension for 2 min at 72°C). PCR products (10 μl) were electrophoresed in a 2% agarose gel and visualized using ethidium bromide. Fragments of expected sizes were obtained (330 base pair for IL-6 and 575 base pair for GAPDH).

Quantitation of PCR products, determination of linear range with reference to number of PCR cycles and amounts of cDNA subjected to PCR and calculation procedures have been described previously (Henninger *et al*, 1993). The PCR products were subjected directly to ion exchange chromatography connected to an on-line UV spectrophotometer (Gynkotek, Germering, Germany) that allowed quantitation of PCR products at 260 nm.

Photoreactivation and detection of CPD in KB and NHEK To allow for uptake, duplicate cultures of KB cells or NHEK were preincubated for 1 h with a solution of Photosomes (20 μg per ml, *Anacystis nidulans* photolyase encapsulated in liposomes) (Applied Genetics, Freeport, NY) in 1 ml MEM supplemented with 0.5% fetal calf serum or KBM-medium, respectively. Different batches of photosomes were made available for experiments with KB and NHEK. The batch used for experiments on KB was preservative free, whereas the batch used on NHEK contained 0.97% phenoxyethanol as a preservative. The presence of phenoxyethanol reduced both basal and UVC induced levels of IL-6 release. After dialysis to remove the preservative, both basal and UVC induced IL-6 release in the presence of photosomes was restored; however, this procedure also led to loss of photolyase activity and consequently, for experiments in NHEK, we used photosomes with phenoxyethanol present. After irradiation with 20 J UVC per m^2, cells were kept in the dark or illuminated with PRL. A 6 mm glass plate was placed on top of each Petri dish, and cells were irradiated from above for 20 min with a bank of two fluorescent broad spectrum Thorn Atlas UVA lamps with peak emission at 365 nm and irradiance of 4.7–5 W per m^2 (total PRL fluence, about 6 kJ per m^2). One set of dishes for each treatment was incubated for 24 h and supernatants collected for analysis of IL-6 concentration. The second set was trypsinized and fixed onto glass microscope slides for immunostaining of CPD as described previously (Clingen *et al*, 1995b) using the H3 mouse monoclonal antibody with high specificity for TT and TC CPD (Roza *et al*, 1988). After immunostaining, cells were viewed under a light microscope at ×400 magnification and images recorded using a color CCD camera and an ImageDok color image analysis system (Kinetic Imaging, Liverpool, U.K.). Cell nuclei of 50 individual cells were marked out for each treatment in separate experiments and the average intensity (red chromaticity) of the chromogenic substrate napthol-AS-MX-phosphate/fast red determined. As controls KB and NHEK cells were treated with photosomes or PRL alone. Additional controls were performed with NHEK in which the PRL with or without photosomes was given before UVC irradiation.

Statistical analysis Data for IL-6 production are presented as the mean and its standard error from at least three separate experiments. Slopes for dose–response curves for IL-6 release were determined over the linear part of the curve by multiple regression allowing for differences in basal release between experiments. Comparisons of photosome treatments (**Figs 5**, **6**) were made by paired t test on log transformed data.

RESULTS

IL-6 release by KB cells is an active process Preliminary time course experiments were performed with 5, 10, and 20 J 254 nm UVC per m^2. There was a significant induction of IL-6 secretion with

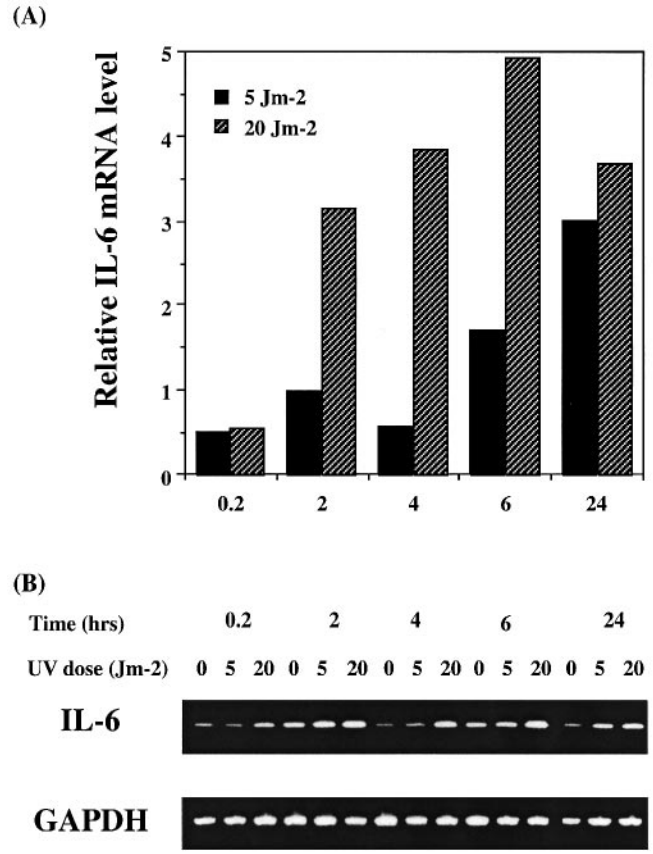

Figure 1. IL-6 mRNA levels are induced by UVC. (*A*) mRNA levels in KB cells following irradiation with 5 (■) or 20 (▨) J UVC per m^2. The results (mean of two separate experiments) are expressed as the ratio of the absorbance of the IL-6 PCR product to the corresponding GAPDH PCR product and are normalized to the unirradiated sample. (*B*) Representative agarose gel of a time course experiment. Note that quantitation in (*A*) was performed by ion exchange chromatography, not by gel densitometry.

all doses 24 h after irradiation (data not shown). To determine whether IL-6 release was an active process or a result of passive diffusion of preformed IL-6 from dying cells we investigated the effect of UVC (5 and 20 J per m^2) on the levels of IL-6 mRNA (**Fig 1**). Following irradiation at 254 nm, the level of mRNA was significantly upregulated after 2 h with 20 J per m^2 and after 6 h with 5 J per m^2. Time course experiments for both UV-induced IL-6 release (**Fig 2*A***) and number of live cells assayed by trypan blue were studied (**Fig 2*B***). Following 20 J 254 nm irradiation per m^2 a significant induction of IL-6 protein was observed after 2 h (**Fig 2*A***), which plateaued after 5 h. In contrast a significant difference in cell number between the unirradiated and irradiated treatments was not evident until between 12 and 24 h (**Fig 2*B***).

Wavelengths in the UVC/UVB range are most effective in inducing IL-6 release KB cells and NHEK were irradiated at the mercury emission lines 254, 302, 313, 334, and 365 nm using a monochromator. For each wavelength selected, a dose–response curve was generated (**Fig 3*A*–*E***). After 24 h in culture, the basal production of IL-6 was on average 300 pg per ml for KB cells and 400 pg per ml for NHEK. In each experiment, a net increase of IL-6 in the supernatants was observed. Clearly, the most effective wavelength for induction of IL-6 secretion for both cell types was 254 nm. The level of induction was very similar between KB and NHEK: a 12–13-fold induction of IL-6 was observed with 40 J per m^2 at 254 nm as compared with 6–7-fold induction with 1054 J per m^2 at 302 nm and 1.6 fold with 14.4 kJ per m^2 at 313 nm. Lower but significant levels of IL-6 secretion were observed at wavelengths of 313 nm and above (**Table I**).

In separate experiments KB cells were irradiated with doses up to

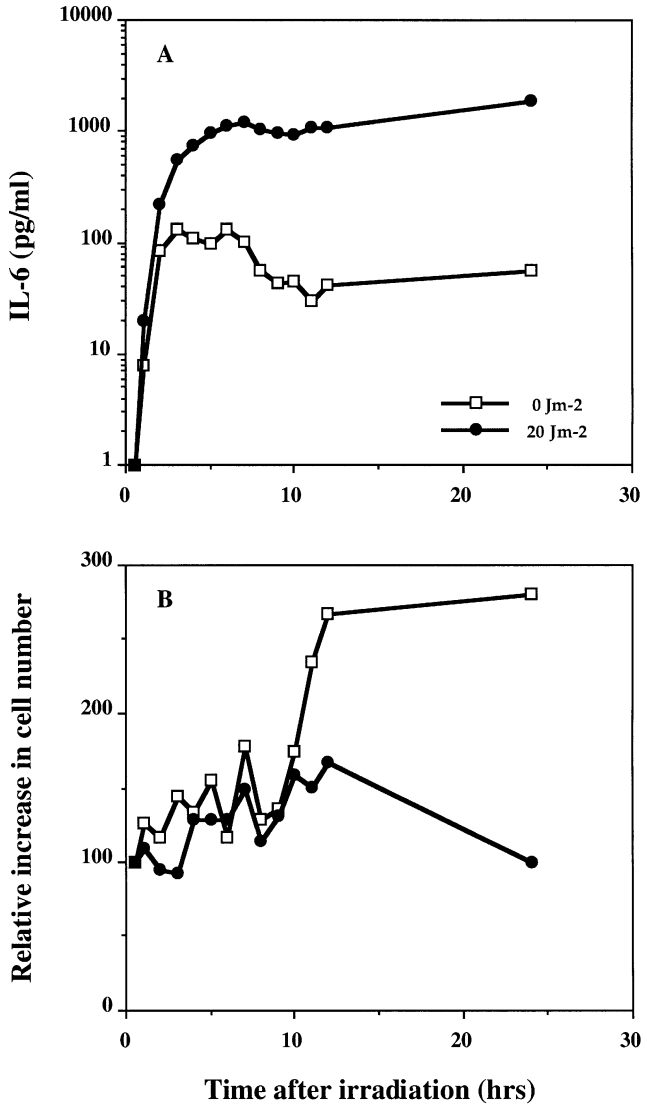

Figure 2. UVC-induced IL-6 secretion precedes cytotoxicity. KB cells were irradiated with 0 (□) or 20 (●) J UVC per m^2. At indicated times, supernatants were collected and the concentration of IL-6 was determined by ELISA. For each time point, the number of living cells was determined using trypan blue. Results from one typical experiment are expressed as concentration of IL-6 (*A*) or as the relative increase over initial cell number (*B*).

64.8 kJ per m^2 at 313 nm and 38 kJ per m^2 at 335 nm. These gave only 3- and 2.6-fold induction of IL-6, respectively. In terms of cytotoxicity, 64.8 kJ per m^2 at 313 nm was equitoxic to 40 J per m^2 at 254 nm, which gave approximately a 12-fold increase in IL-6 release, suggesting again that the UV-induced release of IL-6 and cytotoxicity were not directly correlated.

Wavelength dependence of IL-6 induction correlates with the DNA absorption and CPD action spectra For each dose-response curve, the rate of IL-6 induction per J per m^2 was calculated by regression analysis over the fluence range inducing a linear increase in secretion of IL-6 (**Fig 3**). The rate of IL-6 production at each wavelength is shown in **Table I**. The relative rate of IL-6 production for KB and NHEK, together with the relative rate of CPD formation (Matsunaga *et al*, 1991), and relative DNA absorption (Sutherland and Griffin, 1981) at the same wavelengths are plotted in **Fig 4**. From these graphs it is evident that the wavelength dependence for the three endpoints are very similar, suggesting the involvement of DNA damage in UV-induced release of IL-6.

Photoreactivation of CPD correlates with inhibition of UVC-induced IL-6 release in NHEK cells In our initial experiments,

KB cells were preteated with photosomes (PS), irradiated with 20 J UVC per m^2 and then exposed to 6 kJ PRL per m^2. This treatment significantly reduced the yield of CPD (54% reduction, four experiments, $t = 4.6$, $p < 0.02$) (**Fig 5*A***), and IL-6 release (31.1% reduction, four experiments $t = 4.9$, $p < 0.02$) (**Fig 5*B***). Photosomes on their own did not affect the yield of CPD (**Fig 5*A***), or UV-induced cytokine release (**Fig 5*B***); however, in these KB cells, irradiation with 6 kJ PRL per m^2 in the absence of photosomes significantly reduced IL-6 production (**Fig 5*C***), but not CPD formation (data not shown). Therefore, there was no definite evidence that induction of cytokine in KB cells was associated with CPD formation.

We next investigated whether or not there was a similar effect of PRL on the untransformed NHEK. In these cells the same dose of PRL (6 kJ per m^2) did not significantly affect dimer yields (**Fig 6*A***) or IL-6 production (**Fig 6*B***) in the absence of photosomes.

In these later experiments the batch of photosomes contained the preservative phenoxyethanol that reduced both basal and induced levels of IL-6 release (**Fig 6*B* *versus* *D***), but did not prevent photoreversal of CPD formation. When NHEK were preincubated with these photosomes, irradiated with 20 J UVC per m^2, and then subjected to PRL, there was a 57.3% reduction in the yield of CPD (**Fig 6*C***). The same treatment gave a 55.7% reduction in UVC-induced IL-6 release ($p < 0.003$) (**Fig 6*D***). If the NHEK were treated with photosomes alone, or with PRL alone, or if the UVC irradiation was given after PRL, with or without photosomes, then no significant reduction in the IL-6 induction and no reduction in the yield of CPD was observed (**Fig 6*A–D***).

DISCUSSION

In this study the induction of IL-6 by UVR was maximal at 254 nm in both the transformed keratinocyte cell line KB and the primary NHEK. Irradiation at 302 nm was ≈60–70 times less effective at inducing IL-6 and wavelengths beyond 313 nm induced lower but still significant levels.

Significant cell death occurred following irradiation at those wavelengths and doses most efficient at inducing IL-6. This might have suggested that the increase in IL-6 in the supernatant was by passive release from dead cells, but time course experiments showed that IL-6 mRNA and protein were induced well before any effect on cell number was observed. We also determined the levels of preformed IL-6, by separating KB cells from the supernatant and preparing cell lysates. We found no evidence of higher levels of IL-6 in lysates prepared from unirradiated cells (data not shown).

The similarity between the wavelength dependence of IL-6 release,

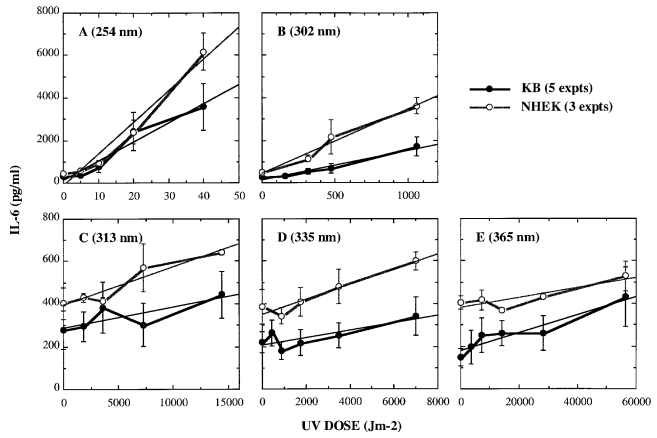

Figure 3. Wavelength dependence of IL-6 induction in KB cells and NHEK. Cells were irradiated at different fluences with a monochromator at 254 nm (*A*), 302 nm (*B*), 313 nm (*C*), 334.9 nm (*D*), and 365 nm (*E*). Supernatants were collected after 24 h and the concentration of IL-6 was measured by ELISA. Each data point shows the absolute increase in levels of IL-6 and represents the mean of five separate experiments (±SEM) for KB (●) and three separate experiments (± SEM) for NHEK (○). Best fit regression lines are shown.

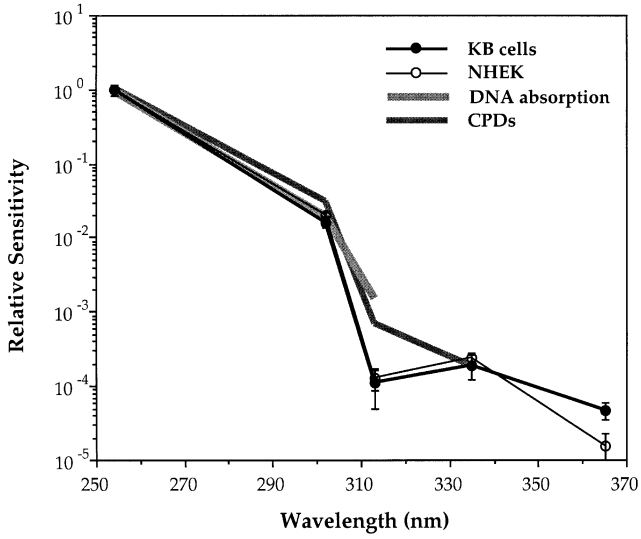

Figure 4. Wavelength dependence of IL-6 induction in KB cells and NHEK matches the spectra of DNA absorption and CPD formation. The rate of IL-6 induction was derived from the linear portion of the dose–response curves by regression analysis (**Fig 3**). Each data point represents the mean (±SEM) of five or three separate experiments for KB (●) and NHEK (○), respectively. For comparison, the relative DNA absorption and the relative rate of CPD formation at the same wavelengths are also shown (Sutherland and Griffin, 1981; Matsunaga *et al*, 1991).

Table I. Rate of IL-6 induction after 24 h at 254, 302, 313, 334, and 365 nm in KB and NHEK cells[a]

Wavelength (nm)	IL-6 (pg per ml per Jm^2)	SEM	No. experiments	Degrees freedom	t	P
KB						
254	89.6	±14.7	5	19	6.07	<0.001
302	1.42	±0.20	5	19	7.12	<0.001
313	0.00988	±0.00557	5	19	1.77	<0.1
335	0.0172	±0.0064	5	24	2.68	<0.02
365	0.00419	±0.00103	5	24	4.06	<0.01
NHEK						
254	149.1	±13.7	3	11	10.86	<0.001
302	3.035	±0.463	3	8	6.55	<0.001
313	0.0188	±0.0046	3	11	4.04	<0.01
335	0.0353	±0.0078	3	11	4.50	<0.001
365	0.00234	±0.0092	3	11	2.56	<0.05

[a]The rate of IL-6 induction was derived from the linear portion of the dose–response curves (**Fig 3**) by regression analysis. Each value is determined from five separate experiments for KB and three separate experiments for NHEK and shows the SEM.

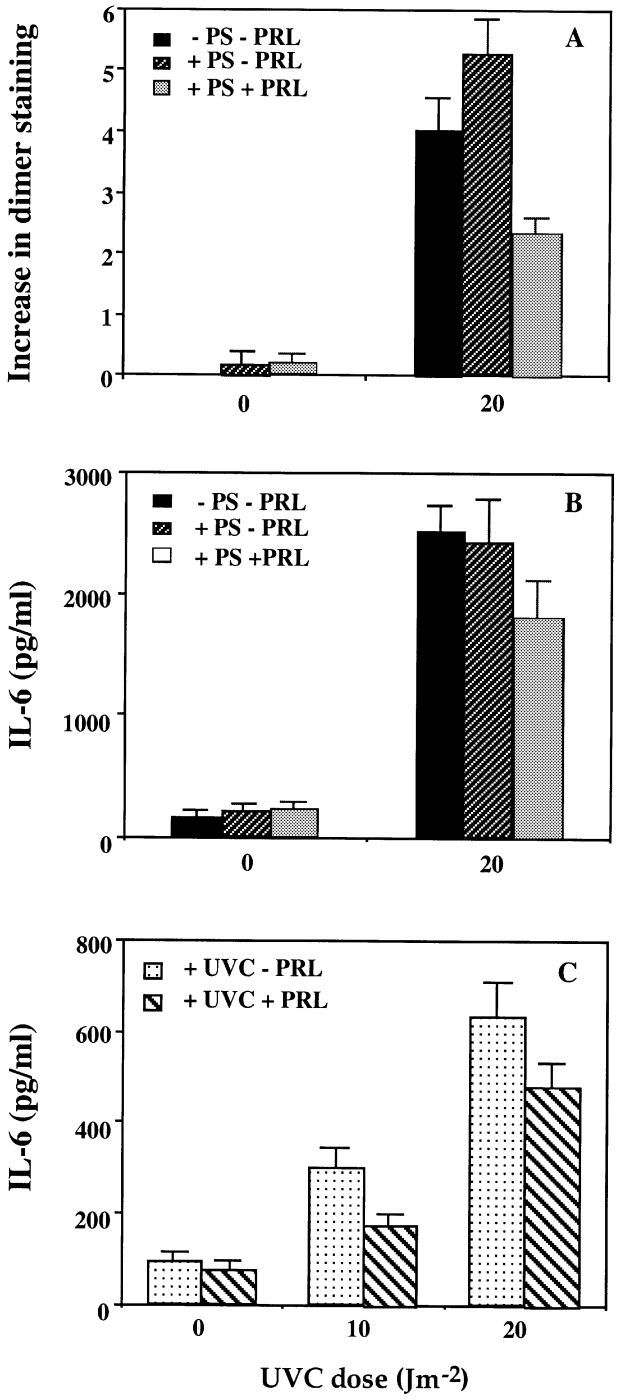

Figure 5. Photoreversal of CPD and UV-induced IL-6 release in KB cells. ■, Cells not pretreated with PS, irradiated with 0 or 20 J UVC per m^2, and not exposed to PRL; ▨, cells incubated with PS, irradiated with 0 or 20 J UVC per m^2, and not exposed to PRL; ▩, cells incubated with PS, irradiated with 0 or 20 J UVC per m^2, and exposed to PRL. (*A*) CPD: the +PS+PRL group shows a signficant reduction in dimer staining ($t = 4.6$, $p < 0.02$) as compared with the +PS–PRL group. (*B*) IL-6 release: the +PS+PRL group shows a significant reduction in IL-6 release ($t = 4.9$, $p < 0.02$) as compared with the +PS-PRL group. Results of (*A*) and (*B*) are the mean of four independent experiments (±SEM). (*C*) Effect of PRL on IL-6 release in the absence of photosomes (four experiments). Cells not pretreated with PS, were irradiated with 0, 10, or 20 J UVC per m^2, and not exposed (▩) or exposed (▧) to PRL. The –PS+PRL group shows a significant reduction in IL-6 release as compared with the –PS–PRL group at 10 J per m^2 ($t = 3.93$, $p < 0.05$) and 20 J per m^2 ($t = 3.77$, $p < 0.05$).

CPD formation, and DNA absorption suggest that DNA damage induces the release of IL-6 from human keratinocytes. Similar action spectra generated for UV-induced gene expression (Stein *et al*, 1989) and immunosuppressive effects (Hurks *et al*, 1995) have been used to postulate that such effects are mediated by DNA damage and in particular by the formation of CPD or (6–4) photoproducts.

To investigate the type of DNA damage involved in IL-6 release we used photosomes to reverse the CPD formed following 254 nm UVC irradiation. Results obtained using the transformed KB cells suggested that photoreversal of CPD might reduce the induction of IL-6 by UVR; however, PRL itself reduced UVC-induced IL-6 secretion in these cells, even in the absence of photosomes. This effect of PRL did not involve an endogenous photoreactivating enzyme, because no reduction in CPD was observed. Other studies have shown that UVA can affect UVB-induced immunosuppressive endpoints. An immunoprotective effect has been described by Reeve *et al* (1998) in hairless mice in which PRL prevented the inhibition of contact hypersensitivity induced by UVB and solar simulated radiation. These mice do not possess a photoreactivating enzyme, and the authors suggested that the PRL induces some unidentified immunoprotective product that appears to inhibit the UVB-induced immunosuppression. Any *in vivo* relevance of a UVA photoprotective effect against UVB induced immunosuppression or cytokine production remains to be established.

In primary untransformed NHEK and at the fluences used here, PRL on its own did not affect IL-6 release. Reduction of IL-6 release was specifically associated with photoreversal of CPD, and was seen only when NHEK were pretreated with photosomes, irradiated with UVC, and then subjected to PRL. This, together with the wavelength dependence, implies an important role for DNA damage and particularly for CPD in triggering IL-6 release at short UVC and UVB wavelengths.

The results presented in this study are consistent with the increasing body of data that indicate that unrepaired CPD can trigger the release of cytokines from human and rodent cells. These cytokines may in turn

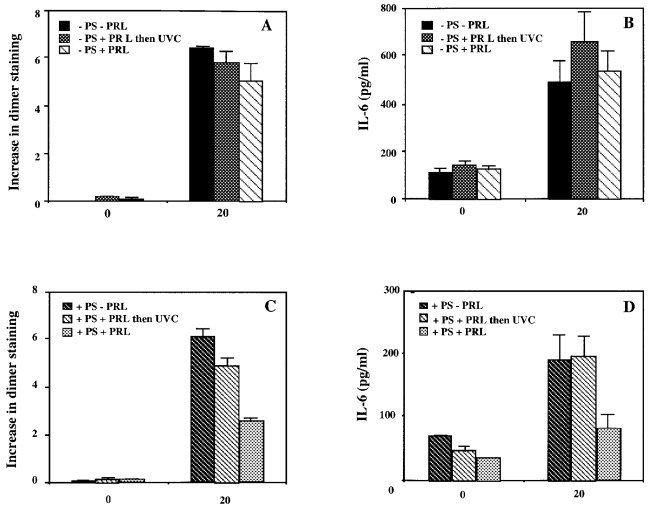

Figure 6. Photoreversal of CPD suppresses UV-induced IL-6 release in NHEK cells. (*A*) Cells not pretreated with PS, dimer staining; (*B*) cells not pretreated with PS, IL-6 release; (*C*) cells pretreated with PS, dimer staining; (*D*) cells pretreated with PS, IL-6 release. ■, Cells not pretreated with PS, irradiated with 0 or 20 J UVC per m^2, no PRL; ▩, cells not pretreated with PS, exposed to PRL, then irradiated with 0 or 20 J UVC per m^2; ▧, cells not pretreated with PS, irradiated with 0 or 20 J UVC per m^2, then exposed to PRL; ▨, cells incubated with PS, irradiated with 0 or 20 J UVC per m^2, and not exposed to PRL; ▧, cells incubated with PS, exposed to PRL, then irradiated with 0 or 20 J UVC per m^2; ▥, cells incubated with PS, irradiated with 0 or 20 J UVC per m^2, and then exposed to PRL. The UVC irradiated +PS+PRL group shows a significant reduction in dimer staining (*C*, $p < 0.003$) and in IL-6 release (*D*, $p < 0.003$) as compared with the UVC irradiated +PS–PRL group. The UVC irradiated –PS+PRL group showed no significant reduction in dimer staining (*A*) or IL-6 release (*B*) compared with the –PS–PRL group. Results are the mean of three independent experiments (±SEM).

affect erythema, inflammatory, or immunologic responses (Kripke *et al*, 1996; Yarosh and Kripke, 1996). Wolf *et al* (1995) have demonstrated that treatment of mouse skin with the CPD-specific DNA repair enzyme (T4 endonuclease V) encapsulated in liposomes following UVB irradiation partly protected mice against sunburn cell formation. In the marsupial *Monodelphis domestica* (Applegate *et al*, 1985) photoreactivation of CPD almost completely prevents the formation of sunburn cells. In mice, treatment with T4 liposomes reduced the UVB-induced inhibition of delayed and contact hypersensitivity reactions (Kripke *et al*, 1992). Similarly, Nishigori *et al* (1996) have shown that *in vitro* treatment of murine keratinocytes with such liposomes inhibited the UVB-induced production of the immunosuppressive cytokine IL-10 and *in vivo*, the level of IL-10 in the serum is reduced in irradiated mice. Recently, Vink *et al* (1996) have shown that repair of UV-induced DNA damage using the T4 endonuclease V enzyme prevents the impairment of antigen presenting cell function in the draining lymph nodes of irradiated mice. Moreover, photosomes containing the repair enzyme photolyase can restore the antigen presenting activity of dendritic cells in UV irradiated skin (Vink *et al*, 1997). In human cells, studies using fibroblasts from UV-sensitive xeroderma pigmentosum and trichothiodystrophy patients, have demonstrated that the ability to restore normal levels of the immunomodulatory marker ICAM-1 after UVB irradiation is related to the ability to repair CPD (Krutmann *et al*, 1996). Our study similarly indicates that UV-induced IL-6 release in normal epidermal cells is associated with DNA damage, and specifically CPD formation.

Our experiments using two cell systems have shown little evidence of IL-6 induction by mechanisms not involving DNA damage, and are in contrast to the study of Wlaschek *et al* (1994), who found strong induction of IL-6 following UVA irradiation of HeLa cells at doses much greater than those used in this study (300 kJ per m^2 at wavelengths between 340 and 450 nm as opposed to 7 kJ per m^2 at 335 nm and 55 kJ per m^2 at 365 nm).

The mechanisms whereby DNA damage is translated into an appropriate signal to promote production of cytokines, and whereby different types of DNA damage may lead to different signaling responses remain to be determined. One possible clue may come from the dual role of certain repair proteins in DNA repair and transcription (Bootsma and Hoeijmakers, 1993). The importance of this dual function in the regulation of immunologically relevant genes, such as ICAM-1 in UVB irradiated cells, has been demonstrated recently by Ahrens *et al* (1997). Whether a similar mechanism could be involved in the UVR-induced increased transcription of cytokine genes remains to be resolved.

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